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THE GRAY BULB-ROT OF TULIPS CAUSED BY RHIZOCTONIA TULIPARUM (KLEBH.) N. COMB.

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THE GRAY BULB-ROT OF TULIPS CAUSED BY RHIZOCTONIA TULIPARUM (KLEBH.) N. COMB.

H. H. Whetzel and John M. Arthur [1]

The gray bulb-rot of tulips, long known in Holland and Germany, apparently has not until recently (Whetzel and Arthur, 1924) been reported from North America. In April, 1922, Dr. William Crocker, Director of the Boyce Thompson Institute for Plant Research at Yonkers, New York, sent some diseased bulbs from a bed in a near-by garden to the Department of Plant Pathology at Cornell University for diagnosis. Dr. L. M. Massey obtained from these bulbs pure cultures of a sclerotial fungus. These cultures were turned over to the senior author the following June. Their marked similarity to cultures of *Sclerotium tuliparum* Klebahn which he had obtained from Holland some years ago and was still carrying along, seemed to warrant a further investigation of the disease. A cooperative undertaking for this purpose was at once arranged between the two institutions, the results of which are here set forth.

THE DISEASE

HISTORY [2]

This disease was known in Holland at least as early as 1884, when it came to the attention of Wakker (1885:22), who was then engaged in an investigation of bulb diseases for the Dutch Bulb-Growers' Association.

Wakker appears to have been the first to describe the disease, which he designated merely as the "tulpenziekte" (tulip disease). His observations on the symptoms of the disease and the character of the pathogene are extraordinarily clear and accurate.

Ten years later Ritzema Bos (1894:229) began an investigation of the disease, which he found very destructive in certain sections of Holland at that time. Although he refers to the earlier work of Wakker, he appears to regard it as of little importance. In his later report (1903a:178 [p. 23 Centbl.]) he describes symptoms which are undoubtedly those of the gray bulb-rot, but confuses this disease with the Botrytis blight, which he also had before him (Hopkins, 1921:316–317). Ritzema Bos apparently assumes

[1] Biochemist of the Boyce Thompson Institute for Plant Research.

[2] In view of the fact that no previous paper on this disease has appeared in English, and since several of the most important articles cited in this discussion are not readily accessible to American pathologists, a rather full review of the previous literature on the bulb-rot is here presented.

(1903 a: 190) that Wakker also had only the Botrytis blight under consideration when he described his "tulpenziekte."

Because of the failure of tulips to come up in the spring in soil-infested areas, the disease is called by the Dutch growers "wegblijven" (away remaining) and the contaminated areas "kwade plekken" (bad spots) or "kwade grond" (bad ground). From the fact that Wakker does not use these terms in his early description of the disease, Ritzema Bos (1903 a: 191) concludes that the disease is new to Dutch bulb-growers in spite of the assurance of growers in Noordwijk that it had been known to them for more than twenty years.

Klebahn (1904: 18) observed the disease in the spring of 1903 in the botanical gardens at Hamburg, Germany, and began a series of investigations culminating in three papers (1905, 1906, and 1907), the most important contributions on the subject to date. In his early work (1904) he too confused the gray bulb-rot with the Botrytis blight, but he soon discovered that he was working with two distinct diseases. In his later papers he clearly distinguished the gray bulb-rot from the Botrytis blight, giving it the name *sclerotium disease* (1905: 1–18).

As none of the names thus far applied to the disease appear to be entirely satisfactory, the term *gray bulb-rot* is proposed as indicating more definitely its character.

RANGE AND IMPORTANCE

The gray bulb-rot, which seems to have first appeared in Holland in the early eighties, has gradually become widespread and destructive in certain sections of the bulb-growing districts there. That it was of relatively little importance in 1884 seems evident from the meager consideration given it by Wakker (1885), who, however, reports it as sometimes destroying most of the tulips in a bed. Ritzema Bos (1894: 229) reports what was undoubtedly this disease as having been very destructive in scattered localities in the bulb districts during the seasons of 1892 and 1893, and again (1895: 348) in 1894. In his report ten years later on the investigation begun at that time, he intimates (1903 a: 177–178) that the disease had in the meantime become much more general and serious in the bulb districts, and that it was even then becoming a factor in the export trade in bulbs to adjacent countries, because of losses to gardeners in Germany and elsewhere who purchase the bulbs for forcing and outdoor plantings. Since, however, he constantly confused the bulb-rot with the Botrytis blight, one cannot estimate from his reports the relative importance of the two diseases.

Klebahn (1905: 13–14) concluded from his early inoculation experiments that the bulb-rot was far more dangerous and serious than the Botrytis blight, but from observations made shortly afterwards on a visit to the bulb-growing districts of Holland he observed (1907: 3) that the Botrytis blight was not so harmless as he had been led to believe, that it was much more widespread than the bulb-rot, and that it caused the growers much

trouble. There is, however, little doubt that the bulb-rot is a serious disease in regions where it is well established (Klebahn, 1907:12–13).

The disease has been reported from Switzerland (Müller-Thurgau, 1908:750, and Lendner, 1911), and also by Jaczewski from Russia (Diakonoff, 1913:281). Although no reference to the disease in England has been found in the literature,[3] it undoubtedly occurs there; to what extent, however, is not known. The senior author received a culture of the pathogene isolated by Miss Wakefield from tulip bulbs in Kew Gardens in 1922. It is remarkable that it has not previously appeared in America, or, if it has, that it has not sooner attracted attention. With the growing restrictions on the importation of Dutch bulbs, and the possibility that Americans must eventually depend more and more on home-grown stock, the appearance of the disease in this country assumes a more than scientific interest.

The bulb-rot is of greatest economic importance in those districts where bulbs are grown for sale and export, as in Holland. Even there it appears to be confined to certain districts (Klebahn, 1907:12–13). Since the pathogene appears to be transported but rarely on bulbs in commerce and is apparently not disseminated by spores, it is only occasionally destructive in tulip beds grown for blossoms, and its ravages are therefore largely confined to the beds that became contaminated by the original importation. Klebahn (1904:21–22), reports, as a result of a survey of the tulip plantings about Hamburg, that the disease is rarely met with, and ther only in an occasional planting of bulbs for flowers. The fields of the one grower near Hamburg who produced bulbs for the trade were free from the disease. Klebahn reports a few isolated cases of the disease in tulips imported from Holland into other parts of Germany (1907:4).

The gray rot appears, therefore, to be a disease of relatively little danger to tulip-growing in America, where flower production is at present the chief object, even though the bulbs continue to be imported from bulb-growing countries such as Holland, where the pathogene is well established. Should a tulip-bulb-growing industry develop in the United States, the infrequent transportation of the pathogene on bulbs, the slowness with which it spreads from bed to bed, and the ease with which contaminated soil may be disinfected, does not warrant any particular concern as to the continued importation of Dutch bulbs into this country.

SYMPTOMATOLOGY

The first evidences of the disease are the bare spots in the tulip beds in the spring, where the plants fail to come up. Nearly all bulbs in the soil-contaminated area are usually so injured that they fail to grow. When

[3] The reference to this disease by Bewley in his book *Diseases of Greenhouse Plants* (1923:71) is an error. He has mistaken a Botrytis disease (not caused by *B. tulipae*) for the sclerotium disease, as indicated by cultures of his fungus which he sent to the senior author. His citation of the article by Ramsbottom is also an error, for that deals only with the nematode of hyacinths.

affected bulbs do send up leaves, their growth is greatly retarded. They soon die and wither away, usually before they can expand, because the fungus gradually rots them off underground. Initial infection evidently occurs in the fall and early winter shortly after the bulbs are put out into the beds, or early in the spring. Observations by the authors on the symptoms of the disease check fully with the description and illustrations of them given by Klebahn (1905, figures 4–5, and 1906, figures 53–55).

When diseased bulbs are dug up, they are found to be more or less rotted, the infection beginning usually at the tip, or nose, of the bulb. The roots are in most cases abundant and healthy (Plate I). The leaves and the flower shoot, if they have started, are rotted, and the adjacent bulb scales within are more or less involved. The rot is a dry one, the tissue for a time remaining rather firm (Plate II). The healthy white tissue is turned to a grayish or a reddish gray color, a very distinctive symptom of this disease.

On digging up the diseased bulbs, one is at once struck by the fact that the soil clings tenaciously to the exterior of the rotted parts. This is evidently due to the external mycelium, which binds the soil particles together and holds them to the rotted bulb scales. Embedded in the soil about the bulb are numerous, more or less globose, dark brown bodies, the sclerotia of the pathogene. These, when dry, become almost black. They vary from 1 to 9 millimeters in diameter. They are often present also in large numbers on the surface of the rotted scales and neck of the bulb under the adhering soil (Plate I). The sclerotia are but loosely attached to the surface of the diseased tissues, and come away easily with the removal of the soil. Some are occasionally to be found within the rotted bulb between the decayed scales. There is an abundance of grayish white mycelium on the outer surface of the bulb covering the diseased parts (Plate I). Within the bulbs this mycelium frequently forms a felty layer between the diseased scales (Plate II).

ETIOLOGY

Identity of the pathogene

The cause of the gray bulb-rot is a fungus named by Klebahn (1905:13) *Sclerotium tuliparum*. Wakker (1885) appears to have been the first investigator to discover and describe this pathogene, though he did not give it a name. He, however, pointed out that it differs fundamentally in many features of its structure from *Peziza* (*Sclerotinia*) *bulborum*, the cause of the black rot of hyacinths, which he at that time had under investigation. Ritzema Bos (1903 a:182), who next investigated the disease, confused the causal organism with *Botrytis tulipae* (Lib.) Hopkins.[4] He

[4] Hopkins (1921:328–331) has shown *Botrytis parasitica* Cav. to be identical with *Sclerotium tulipae* Libert, and has made the combination *Botrytis tulipae* (Lib.) Hopkins.

mentions the earlier work of Wakker, but attributes the disease there described to *Botrytis tulipae* also. It is evident from his description of the symptoms (1903a:178–181) that he was dealing with bulbs affected with *Sclerotium tuliparum*, as well as some attacked by *Botrytis tulipae*. This conclusion is further supported by his observations (1903a:186–187) on the two types of sclerotia and mycelia which he found in the diseased bulbs.

Klebahn (1904:19–21), investigating the diseases of tulips in the gardens in Hamburg in the spring of 1903, described the symptoms of the gray-rot disease and, following the lead of Ritzema Bos, likewise attributed it to *Botrytis tulipae*, with which he made infection experiments. The large sclerotia which he found in the soil and about the crowns of rotting tulips, and which failed to develop in his cultures of *Botrytis tulipae*, led him to doubt their identity with the small sclerotia of this species as is shown by the following: "Diese Umstände zeigen allerdings zunächst nur, dass zu der *Botrytis* überhaupt Sklerotien gehören, aber nicht unbedingt, dass die an den krankeu [*sic*] Zwiebeln aufgefundenen grossen Sklerotien (Fig. 3) mit der auf den Blättern vorkommenden Botrytis in Zusammenhang stehen." (Klebahn, 1904, figures 1 and 3.)

Continuing his investigations, Klebahn soon (1905:14–17) came to the conclusion that the large sclerotial fungus with which he was working is distinct from *Botrytis tulipae* as well as from *Sclerotinia bulborum*, since he was unable to obtain any conidial or sexual fruiting structures. His later investigations confirmed this conclusion but disclosed nothing further as to the taxonomic relationships of the fungus. He named it *Sclerotium tuliparum* (1905:13), apparently being unaware that this name had already been applied by Schlechtendal (Bouché, 1831:223) to the fungus now known as *Botrytis tulipae*.

The fungus associated with the rotting tulip bulbs in the beds near the Boyce Thompson Institute corresponds in all respects, both in nature and in pure culture, with the descriptions and illustrations given by Klebahn. Moreover, comparisons of pure cultures with cultures of the fungus received from Holland[5] and England[6] show only such slight cultural differences (Plate III) as are to be attributed to physiological variations of individual strains. There appears, therefore, to be no doubt that we are here dealing with the pathogene now commonly known to European workers as *Sclerotium tuliparum* Klebahn.

Taxonomic relationships

In the course of these investigations, certain distinctive characters in the morphology of the pathogene have been observed which seem to

[5] Three strains were isolated from sclerotia on bits of tulip bulbs sent to the senior author by Ritzema Bos in 1915. Another culture from Holland was obtained from the pure-culture laboratory of Dr. Westerdijk at Baarn in 1924. All of these were identified by the senders as *Sclerotium tuliparum* Klebh.

[6] The culture from England isolated by Miss Wakefield from tulips at Kew Gardens was sent to the authors from the Lister Institute, Chelsea Gardens, London; their number, 1497.

afford a more satisfactory taxonomic placing of the fungus than in the form genus Sclerotium, where it was left by Klebahn.

In pure cultures on potato agar, the fungus forms an appressed white mycelium which is inclined to a radiate rhizomorphic formation (Plate III, A). The medium is soon discolored, often taking on a distinctly reddish tinge which deepens with age. The sclerotia first appear as irregular, white, cottony masses on the surface of the culture, in a broad zone at some distance from the center of the colony (Plate III, A, B, and C). They soon turn to a pale yellowish buff, deepening to a dark reddish brown, and eventually become almost black when dry. The sclerotia vary greatly in size and form. They are, in general, globose to loaf-shaped bodies, 1 to 9 millimeters in diameter, and in many cases several are agglomerated into a large, irregular mass. The surface of the sclerotium is dull, fibrillose, and irregular, as contrasted with the smooth, naked, polished surface of the sclerotia of Botrytis or Sclerotinia.

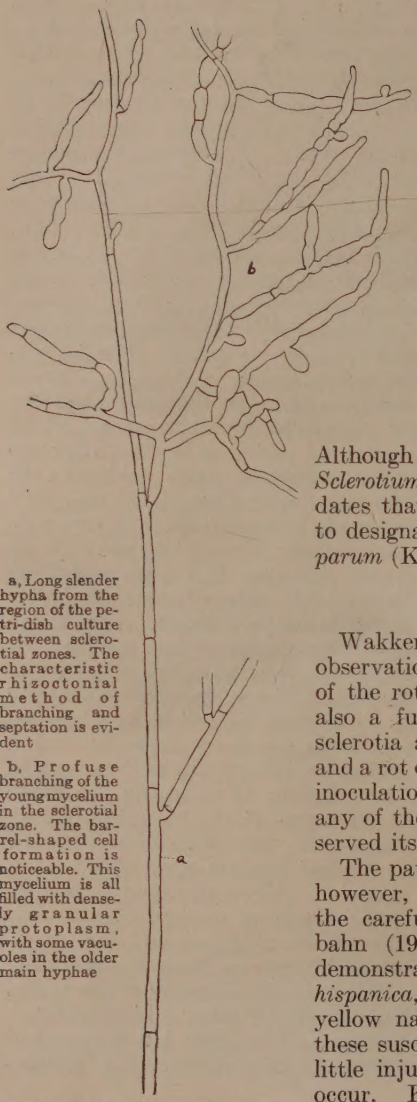
A cross section through a sclerotium shows the interior to be light yellowish brown in color, but of a much lighter tint than the outer narrow zone forming the rind. This colored medulla is in striking contrast to the white medulla of the sclerotia of *Sclerotinia bulborum* or *S. sclerotiorum* (Plate IV, A and D), to which these sclerotia bear a superficial resemblance. A microscopic examination of thin sections of the sclerotia show further marked differences, which Wakker (1885:23) first recognized and pointed out, but which Klebahn appears to have overlooked. Instead of the narrow, thick-walled, interwoven hyphae which constitute the medulla of the sclerotia of the Sclerotinias (Plate IV, D), one finds large, irregularly globose, thin-walled cells forming a typical pseudoparenchyma (Plate IV, A). Instead of the distinctly differentiated rind of thick-walled, black cells forming the rind of the sclerotia of the Sclerotinias, there is here a pseudorind differing in structure from the medulla only in that the cells are generally more elongated and the walls are darker in color (Plate V, B). As the sclerotium matures and dries, the cells of the pseudorind collapse (Plate V, C). The transition from medulla to rind is gradual, and is marked only by an increasingly deeper tinting of the cell walls. Covering the young sclerotia and arising from the cells on the outside is a loose, wefty covering of septate hyphae, the cells of which are sometimes slightly barrel-shaped and are branched in the manner characteristic of Rhizoctonias. This fibrillose mycelial coating is very abundant in the early stages of the development of the sclerotium (Plate V, A), but collapses and disappears more or less as the sclerotium matures. The walls of this mycelium also become reddish brown with age, but are of a lighter tint than the cells of the pseudorind.

A comparison of the sclerotial structure of *S. tuliparum* with that of *Corticium vagum* (*Rhizoctonia solani*) and *Corticium stevensii* indicates at once its taxonomic relationships. In the early stages of development of

the sclerotia, their fibrillose coating (Plate V, A) and the color changes through which they pass remind one strongly of the sclerotia of both *C. vagum* and *C. stevensii*. They differ from these species, however, in several distinct respects. They are compact and definite in form like the sclerotia of *C. stevensii*, instead of diffuse and crust-like as are the sclerotia of *C. vagum*, though some other species of Rhizoctonia produce very definite, more or less globose, sclerotia (Matz, 1921:15). The internal structure is likewise different in certain respects. The cells of the medulla are more globose and compact, showing little of the branched hyphal character of the cells of the medulla of *C. vagum* and *C. stevensii* (Plate IV, B and C). The large intercellular spaces so evident in the medulla of *C. vagum* are almost entirely wanting. The cell walls are much less darkly colored, and there is a distinct pseudorind which is entirely wanting in *C. vagum*. If the structure of the sclerotium of *S. tuliparum* is compared with that of *C. stevensii*, a somewhat more marked similarity is found. The description of the structure of the sclerotium of *C. stevensii* as given by Stevens and Hall (1909:53) does not correspond exactly with the findings of the present authors in an examination of specimens of the fungus sent to them from North Carolina by F. A. Wolf. Stevens and Hall say: "They are entirely devoid of any special epidermal structure, false epidermis, or rind, the peripheral structure being identical with that of the interior." The present authors find that the sections of *C. stevensii* show distinct evidences of a pseudorind (Plate IV, B), due entirely, however, to the collapse of the peripheral cells and not to a deeper coloration of the walls of these cells. The cell walls of the sclerotial hyphae of *C. stevensii* are much darker than those of *S. tuliparum* and uniformly tinted throughout. It is in the collapsed character of the pseudorind that the two species show a similarity. Stevens' drawing (Stevens and Hall, 1909:53, figure 6) gives the impression of thick walls in the cells of the medulla. The authors found that the sections of *C. stevensii* show the walls to be thin, like those in *C. vagum* and *S. tuliparum*. The cells of the medulla are more elongated (often hyphal) than those of *S. tuliparum*, while the distinct intercellular spaces so characteristic of the medulla of the sclerotia of *C. vagum* are much less pronounced. On the whole, the structure of the sclerotium of *S. tuliparum* approaches more nearly that of the sclerotium of *C. stevensii* than it does that of *C. vagum*, though the last two are more alike.

Furthermore, the characters of its mycelium (figures 1, 2, and 3), and its behavior in culture (Plate III), confirm the writers' opinion that *S. tuliparum* is a Rhizoctonia. The branching of the mycelium and the barrel-shaped cells, especially those of the loose hyphae covering the sclerotia, are typically those of a Rhizoctonia. The reddish coloration of the media by the growing mycelium is also a characteristic of many Rhizoctonias.

No basidiomycetous stage has been discovered thus far, but the marked similarity of the mycelium and the sclerotia to these structures in Rhizoc-



a, Long slender hypha from the region of the petri-dish culture between sclerotial zones. The characteristic rhizoctonial method of branching and septation is evident

b, Profuse branching of the young mycelium in the sclerotial zone. The barrel-shaped cell formation is noticeable. This mycelium is all filled with densely granular protoplasm, with some vacuoles in the older main hyphae

FIGURE 1. FORM AND SEPTATION OF THE MYCELIUM OF R. TULIPARUM FROM POTATO AGAR

tonia species whose sexual stages are known to be Corticiums (in the sense of Burt, 1918) makes it all but certain that, when found, the perfect stage of *S. tuliparum* will prove to be a Corticium.

Both Wakker (1885:25) and Klebahn (1905:9) attempted to obtain a fruiting form of the fungus by overwintering the sclerotia in sand or soil. Neither succeeded. Klebahn at least appears to have expected from these sclerotia the production of apothecia.

The writers' investigations seem to warrant the transfer of the fungus to the genus Rhizoctonia. Although Schlechtendal's use of the name *Sclerotium tuliparum* for *Botrytis tulipae* antedates that of Klebahn, it appears legitimate to designate this pathogene *Rhizoctonia tuliparum* (Klebh.) n. comb.

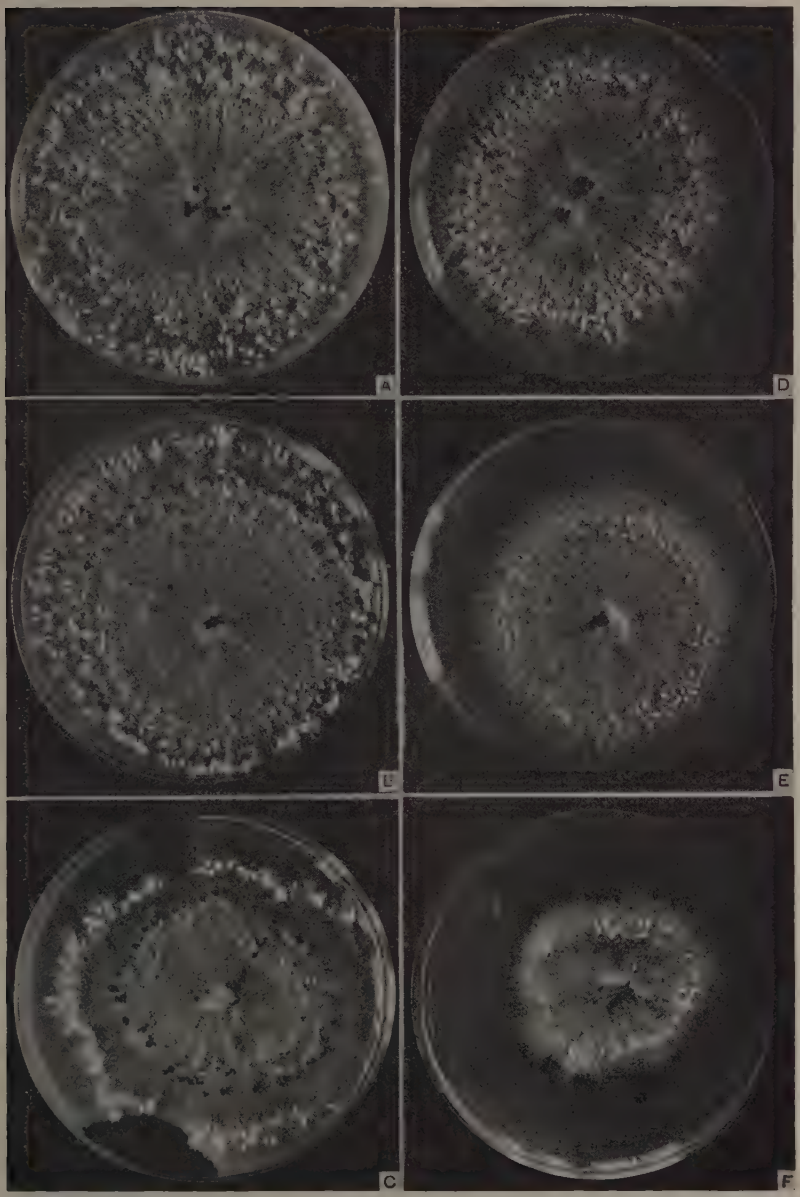
Pathogenicity

Wakker (1885:24–25) concluded from field observations that this fungus was the cause of the rotting of tulip bulbs. He observed also a fungus producing the same sort of sclerotia associated with a rot of hyacinths and a rot of *Dicentra spectabilis*. He made no inoculation experiments with this fungus on any of the three suscepts upon which he observed its occurrence.

The pathogenicity of this fungus on tulips, however, has been fully established through the careful inoculation experiments of Klebahn (1905 and 1907). He (1907:28–29) demonstrated that it may attack also *Iris hispanica*, hyacinths, *Fritillaria imperiales*, yellow narcissus, and *Scilla sibirica*, but on these suscepts, except for the first, relatively little injury and poor sclerotial formation occur. Klebahn observed that the iris is quite as severely injured as the tulip (1906: 564). He was unable to obtain infection on

DISEASED BULBS WASHED FREE OF SOIL, SHOWING SCLEROTIA AND EXTERNAL MYCELIUM

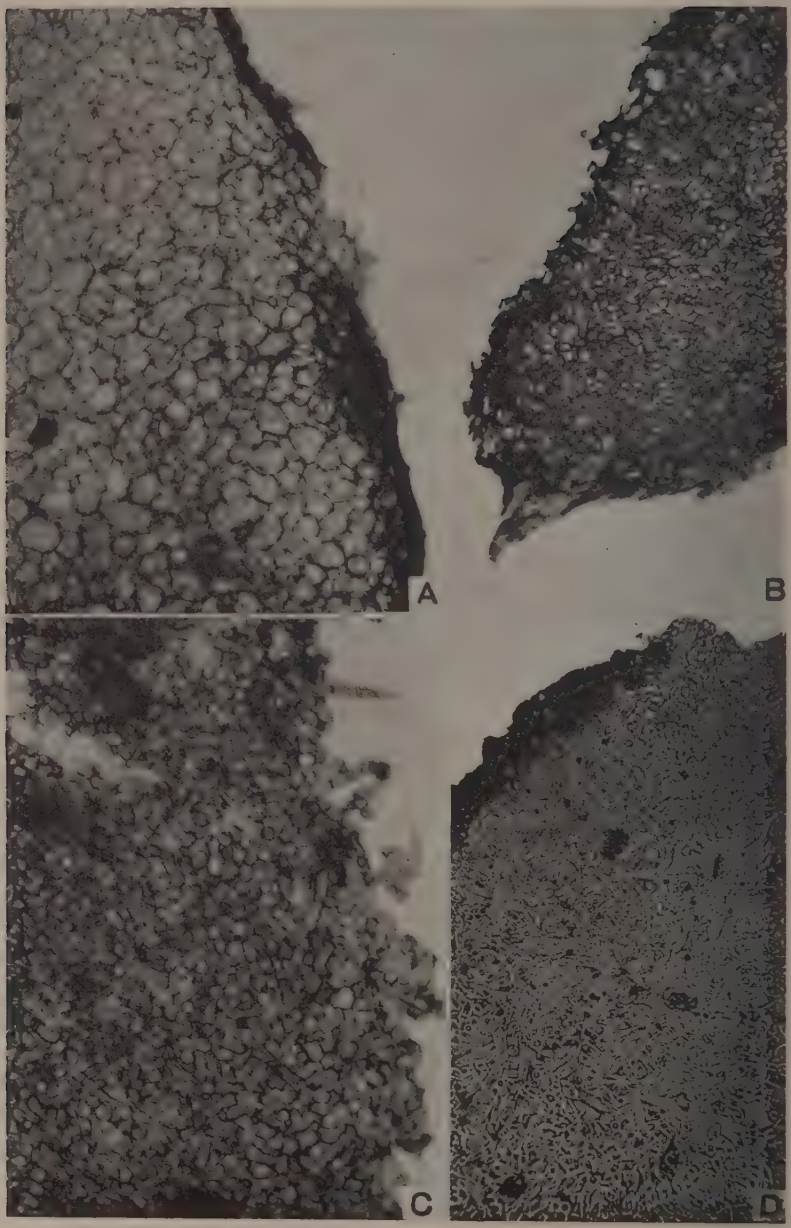
DISEASED BULBS CUT OPEN, SHOWING ROTTED SCALES WITH MYCELIAL FELTS BETWEEN



CULTURES OF THREE STRAINS OF R. TULIPARUM ON POTATO AGAR AT ROOM TEMPERATURE

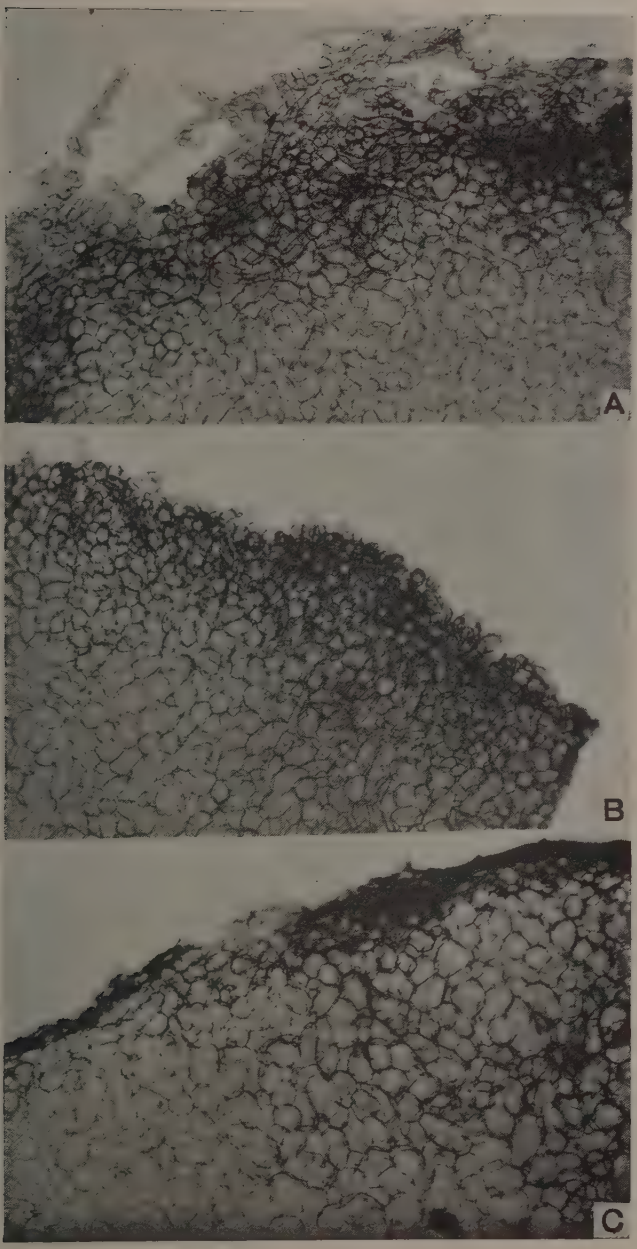
The three plates on the right show growth after ten days, the three on the left the same plates after twenty-three days

A and D, strain (S 195) from Kew Gardens, England. B and E, strain (S 145) from Yonkers, New York. C and F, strain (B xxxv) from Holland. The distinctly slower growth of the Holland strain may be due to the fact that it had been in culture since 1915, while the other two strains were isolated in 1922



FREE-HAND SECTIONS SHOWING THE STRUCTURE OF SCLEROTIA

A, *Rhizoctonia tuliparum*. B, *Corticium stevensii*. C, *C. vagum*. D, *Sclerotinia sclerotiorum*. All × 145



FREE-HAND SECTIONS OF SCLEROTIA OF R. TULIPARUM

A, Young stage of sclerotium from pure culture, showing the external mycelium and the deep coloration of cell walls in the region where the pseudorind is forming. B, Mature sclerotium from pure culture, showing elongated outer cells and deeper-colored walls of the pseudorind; the external mycelial weft is largely torn away. C, Mature sclerotium from bulbs in soil; the cells of the pseudorind have collapsed. All × 145

THREE OF THE SIX PLOTS OF THE 1922–1923 CONTROL EXPERIMENTS

Photograph taken in the spring of 1923, shortly after the tulips were well up. Plot 4 treated with 7½ pounds of formalin; plot 5 untreated (check); plot 6 treated with 10 pounds of formalin

THREE STEAM-STERILIZED PLOTS OF THE 1923–1924 CONTROL EXPERIMENTS

Photograph taken when the plants were in full bloom. The almost perfect stand is noteworthy

TWO OF THE PLOTS OF THE 1923–1924 CONTROL EXPERIMENTS

Photograph taken in the spring of 1924 when the plants were in full bloom. Plot 5 treated with 15 pounds of formalin solution; plot 6 untreated (check). The two crowded outer rows of disease-resistant plants from disease-free bulbs saved from the check plots of 1922–1923 are noteworthy

Muscaria botryoides, *Narcissus poeticus*, *Galanthus nivialis*, and *Crocus vernus*, except in one case on the last-named species. Ritzema Bos (1903 a:181) observed severe losses in iris, hyacinth, and gladiolus in the field, which he attributes to this fungus.

Westerdijk (1911:26), who grew this fungus in pure culture, records failure to obtain infection of the roots of beets, carrots, and potato tubers by inoculation in moist chambers in the laboratory, although it readily attacked tulip and hyacinth bulbs under the same conditions. She obtained infection with it in one case on onion bulbs.

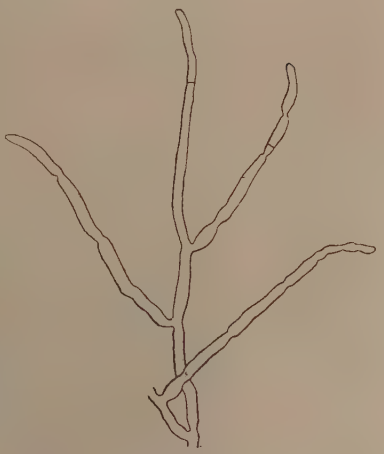


FIGURE 2. BRANCHING TIP OF RAPIDLY GROWING MYCELIUM IN POTATO AGAR, PETRI-DISH CULTURE

The mycelium is filled with densely granular protoplasm

Life history

All the evidence from the work of previous investigators and from the authors' researches indicates that this pathogene depends upon its sclerotia to tide it over from one season to the next. Mycelium which is readily produced from the sclerotia, spreads through the soil and attacks the suscept. The pathogene appears to be a low-temperature parasite, its attack on the bulbs occurring late in the fall or during the mild weather of winter and early spring. Diseased bulbs always show a well-developed root system which is rarely affected. The attack apparently occurs at the nose, or tip, of the bulb as the shoot begins to protrude from the fleshy scales. Because of the restricted range of suscepts, the disease may be expected to become serious only in soils repeatedly planted to tulips. Since affected bulbs are usually more or less completely destroyed before digging time, and since the sclerotia are largely produced in the soil around diseased bulbs, the chances of its rapid dissemination are slight.

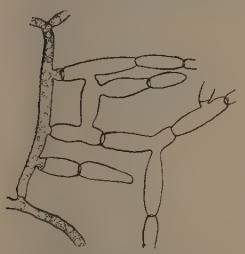


FIGURE 3. BRANCHING AND ANASTOMOSING MYCELIUM IN OLD CULTURE ON POTATO AGAR

Barrel-shaped cells entirely empty; protoplasm of main branch containing granular vacuolate protoplasm

CONTROL

In order to exclude the pathogene from uncontaminated areas, it is important to know in what manner it is transported from one locality

to another. Klebahn (1907:28–29), who gave considerable attention to this matter, concludes that the fungus probably is not often carried on tulip bulbs since infected bulbs are so severely rotted that they would seldom appear in the market. Moreover, he observed that affected bulbs rarely produce bulbils. Both Klebahn and Ritzema Bos suggest that the fungus may be transported in diseased hyacinths or on bulbs of other plants which suffer slightly with the disease. Both Müller-Thurgau (1908:750) and Lendner (1911) cite cases of the occurrence of the disease in Switzerland, in which the pathogene was undoubtedly brought in with diseased bulbs imported from Holland. Lendner observed furthermore that the newly formed bulbils also were attacked. The authors' conclusion on this point, based upon the outbreak of the disease at Yonkers, is that there, at least, all the evidence points to the introduction of the pathogene on the tulip bulbs which were imported from Holland. No other kind of bulbs had been recently planted in these beds. It is indeed probable that the pathogene is only occasionally transported with bulbs in the trade, and this may account for its apparently restricted occurrence. Were it a pathogene commonly transported on tulip bulbs, it would long ago have appeared in America with sufficient frequency and severity to have attracted attention.

The pathogene is a soil-contaminating fungus maintained from year to year by its sclerotia. It appears to spread but slowly year after year from a given center of contamination in the bed, and then only when tulips are planted continuously in the same soil. That it may be disseminated over a plot of ground through spading and cultivation is evident.

The ease with which the sclerotia become separated from the diseased bulbs, their formation in the soil about the bulbs, and the rotting-away of the bulbs before the beds can be reworked and the diseased plants removed, makes sanitary measures such as the removal of diseased bulbs and surrounding soil to appear of little practical value. This seems, however, to be the method generally in vogue among Dutch bulb-growers, who employ for this purpose a special tool (figure 4) known as the Tulpenstecker (tulip sticker).

Rotation where practical is recommended by Klebahn (1907:31), who holds, however, that at least three years must elapse before tulips may safely be returned to soil that has been infested. Moreover, other susceptible bulbs may not be grown therein during the interval (page 26 of same reference).

Ritzema Bos (1903 a:198–202 [p. 350-351 Ztschr.]) tested a number of soil disinfectants for the control of the disease, among these being lime, sulfur, and carbolineum. Of these, only the last proved to be fairly efficient, although a considerable reduction in diseased bulbs was obtained by rolling the bulbs in sulfur after wetting them with a 10-per-cent glycerine solution, together with a liberal use of sulfur placed around the bulbs when planting.

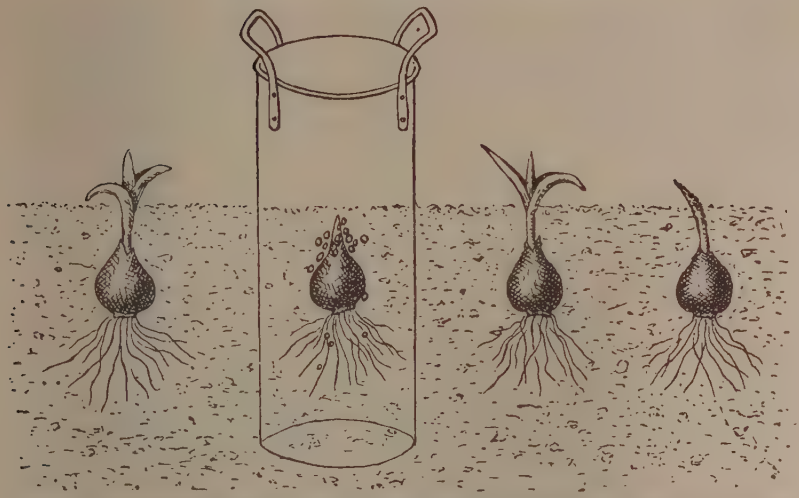


FIGURE 4. "TULPENSTECKER" USED BY THE DUTCH BULB-GROWERS FOR REMOVING DISEASED TULIPS WITH ADJACENT CONTAMINATED SOIL FROM THE BEDS
(From Klebahn, 1907: 30)

The carbolineum was applied at the rate of from 40 to 50 liters to an "are" (=120 square yards), being first mixed with sand which was then applied and worked into the upper layer of soil. When bulbs were planted immediately after treatment of the soil, rather severe injury resulted especially where the larger amount of carbolineum was applied. Where the treated soil was allowed to lie idle until the next season, no injury resulted, and excellent, though not perfect, control was obtained. Ritzema Bos suggested that treatment of contaminated soil early in the summer, allowing it to lie idle until autumn, and then planting to healthy bulbs, would largely obviate injury from the carbolineum. Klebahn (1906: 562) says that in spite of the work of Ritzema Bos and that of a special commission maintained by the bulb growers of Noordwijk, Holland, no wholly satisfactory method of control had at that time been developed. This is, in his opinion, due largely to their confusing this disease with the Botrytis blight.

Klebahn (1907: 32) recommends a combination of the carbolineum disinfection with the removal of diseased bulbs. The few diseased bulbs that appear after soil disinfection can be easily removed early in the spring before the pathogene has spread to any distance in the soil.

The proximity of the contaminated beds at Yonkers to the Boyce Thompson Institute seemed to offer an excellent opportunity to attempt

some experimental work on the control of the disease by means of soil disinfection. The junior author undertook the work of carrying out these experiments in the fall of 1922. The bed in which the experiments were conducted was 66 feet long and 5 feet wide (figure 5). It was bounded on one side by a cement walk and on the other by upright boards about

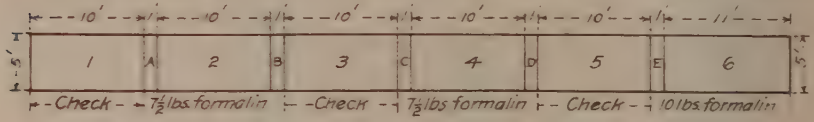


FIGURE 5. PLAN OF THE PLOTS IN THE 1922–1923 CONTROL EXPERIMENTS

6 inches wide. This area was divided into six plots. The first five plots were each 10 feet long, and the sixth was 11 feet long. An isolation strip about 1 foot wide was left between adjoining plots. These isolation strips were set off from the adjacent plots by upright board partitions sunk some inches into the soil. Each plot thus contained 50 square feet of planted space, but, since every other plot (nos. 2, 4, and 6) and its adjoining isolation strips were treated with formalin, the treated areas each contained approximately 60 square feet.

Although the disease had appeared only in certain parts of this bed, in preparing the bed in the spring of 1921 an attempt was made to thoroughly distribute the pathogene before dividing the bed into plots. The diseased bulbs had not been removed in any numbers but had been allowed to rot down, and presumably an abundance of sclerotia were formed.

Plots 1, 3, and 5 (figure 5) were left untreated as checks. To plot 2 with its adjoining isolation strips A and B, and to plot 4 with its adjoining isolation strips C and D, was applied 7½ pounds of 40-per-cent formalin each, diluted with water at the rate of 1 to 50. Plot 6 with its adjoining isolation strip E received 10 pounds of formalin diluted with water at the rate of 1 to 50. The solution was applied as evenly as possible in each case, and the bed was then covered with tarred paper. The soil had been wet by a rain the day before treating.

The formalin was applied on October 11, 1922. The tarred paper was removed on October 23. The bulbs purchased from importers were first peeled, that is, the dry brown scales were removed. All cankers or diseased spots in the fleshy outer scales were carefully cut out, and the bulbs were then disinfected by soaking for one hour in a 1-to-120 solution of formalin, after which they were rinsed through three changes of tap water and spread out to dry.

The bulbs were planted in four rows lengthwise of the plots, none being set in the isolation strips. There were approximately fifty bulbs in each plot. The check plots 1, 3, and 5 were planted first, the treated plots

meanwhile being covered with tarred paper to prevent accidental transfer of contaminated soil to them. The check plots were then covered and the treated plots were planted, after which all plots were left exposed.

On April 17, 1923, the junior author made the following report: "Plot 6 shows practically a complete stand — only two tulips are missing in the entire bed. Plots 2 and 4 show about one-half a stand of tulips. The control plots 1, 3, and 5 do not show more than three or four tulips in each." (Plate VI).

A short time later the senior author inspected the plots and found the visual results even more striking than the report.

It is evident that to effectively disinfect soil contaminated with this pathogene, at least 1 pound of formalin (40-per-cent) must be applied to every 6 square feet of soil. This makes the treatment rather expensive, but it is effective and for small areas is not prohibitive. If the "bad spots" are marked as soon as discovered and are promptly treated, it should rarely be necessary to disinfect large areas. Since steam sterilization should be even more effective, a series of such treatments was made on the same area in the fall of 1923. The experiment (figure 6) was laid out as follows: The plots were the same as those used in the previous season's

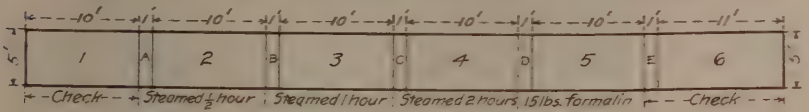


FIGURE 6. PLAN OF THE PLOTS IN THE 1923–1924 CONTROL EXPERIMENTS

(1922) experiments and of the same size and arrangement (figure 5). Before treatment, contaminated soil from the check plots of the previous year was thoroughly mixed with the soil in the other plots. Plots 1 and 6 were left untreated as checks. Plots 2, 3, and 4 were steam-sterilized by the steam-pan method — plot 2 for one-half hour, plot 3 for one hour, and plot 4 for two hours. Plot 5, along with isolation strip D, was treated with formaldehyde solution, 15 pounds diluted in water at the rate of 1 to 50. Four plots were planted with healthy tulip bulbs (variety Moonlight) on November 17, 1923. Plot 5 was not planted until December 3.

On June 10, 1924, the junior author reported that the plants in check plot 1 were badly injured by the disease. Of the 35 bulbs planted in this plot only 13 plants came up, of which 11 showed evidences of disease; 8 of these plants bloomed.

Fifty-two bulbs were planted in each of the steam-sterilized plots. Plot 2 gave a stand of 50 plants, of which only 4 showed disease; 27 bloomed. Plot 3 gave a stand of 50 plants, of which 3 were diseased; 25 bloomed. Plot 4 showed a stand of 47 plants, of which 6 showed disease; 22 bloomed.

The small proportion of blooming plants in these plots in 1923 is to be attributed largely to weak bulbs and not to the disease.

It will be noted that in all three of the steam-sterilized plots (2, 3, and 4) a nearly perfect stand was obtained (Plate VII) and the proportion of diseased plants was approximately the same, 3 to 6 plants to the bed. This indicates that half an hour's steaming is as effective as the longer treatments.

Plot 5, treated with formaldehyde solution, gave a stand of 49 plants, of which 8 showed disease and all blossomed (Plate VIII).

Plot 6, the other check plot, gave a stand of 41 plants out of 52 planted, but of these 21 showed disease; 15 bloomed (Plate VIII). This plot had never shown a high percentage of disease, due apparently to a tile drain across it which tends to keep the soil drier than in the other plots.

These results indicate clearly that the bulb-rot pathogene may be largely eradicated and a good stand obtained by soil disinfection with formalin at the rate of 1 to $1\frac{1}{2}$ pounds to 5 or 6 square feet of soil. The formalin should be diluted in enough water to thoroughly wet down through from 6 to 8 inches of loosely spaded soil. Pan steaming for from one-half to one hour also is effective.

In the control, or check, plots in the spring of 1923, a few plants, as already pointed out, came through with no trace of disease. It appeared possible that these individuals were resistant or immune to the pathogene. In order to test this possibility, these plants were dug after maturity and saved for planting in the contaminated soil in the experimental plots that autumn. The bulbs were planted in two extra rows on either side of plot 6, one of the checks for the experiment of 1923–24. Practically every one of these bulbs grew and produced flowers in the spring of 1924, and again showed no trace of the disease. This indicates rather strongly that they are resistant or immune. Thus a strong probability exists that resistant or immune strains of tulip varieties may be obtained by the selection and propagation of disease-free individuals from populations growing in badly contaminated soil.

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